Article

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Causal Role of Immune Cells in Gastric Cancer: A Mendelian Randomisation (MR) Study

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Background: Observational studies have reported an association between immune cells and digestive diseases. We sought to assess the relationship between immune cells and the risk of gastric malignancy by two-way two-sample Mendelian randomisation (MR) analysis.

Methods: This study used a comprehensive two-sample Mendelian randomisation (MR) analysis to determine the causal relationship between immune cells and gastric malignancy (GC). Based on publicly available genetic data, we explored the causal relationship between 731 immune cell signatures and GC risk. We performed Mendelian randomisation (MR) analyses using genetic variants strongly associated with neutrophil, lymphocyte, eosinophil, basophil and monocyte counts as instrumental variables (IVs). Comprehensive sensitivity analyses were used to verify the robustness, heterogeneity and horizontal pleiotropy of the results.

Results: Two-sample MR analysis revealed that multiple immune cell phenotypes were significantly associated with the risk of gastric malignancy. Among the phenotypes with low uncorrected *p*-values, including CD14- CD16- AC ($p < 0.001$; OR = 0.9730; 95% CI = 0.9598–0.9863); DN (CD4- CD8-) AC ($p < 0.001$; OR = 1.1483; 95% CI = 1.0607–1.2432); IgD on IgD+ ($p < 0.001$; OR = 0.8883; 95% CI = 0.8289–0.9520). Meanwhile, we used Simple mode, Weight median, and Weight mode, all of which led to the same conclusion. Moreover, in our further analysis, gastric malignancy also had a causal effect on the above immune cell types when gastric malignancy was used as an exposure factor, and the results were statistically significant.

Conclusion: The study underscores the crucial role of immune cells in GC development, providing key insights for future research. The statistically significant associations between specific immune cell phenotypes and gastric malignancy risk highlight potential targets for therapeutic interventions aimed at modulating the immune response in GC, thereby opening avenues for precision medicine approaches in the treatment of this disease.

Keywords: immunity; gastric cancer; MR analysis; sensitivity; causal analysis

Introduction

Cancer is an important causative agent of metastatic and invasive malignant tumours and poses a major threat to human health. Gastric cancer ranks among the top five in terms of prevalence and mortality. Helicobacter pylori infection, high sodium diet, and disruption of autoimmune function have been identified as prominent factors underpinning the increased risk of cancer [1,2]. Researchers have actively endeavoured to explore and disseminate a common landscape of gastric malignancy (GC) risk factors. Nonetheless, the complexity of cancer risk remains largely enigmatic and requires continued academic research. Despite serious efforts to improve and manage GC, there are still a considerable number of journals working to alleviate the enormous burden of disease prevalent in the field.

Epidemiological studies have shown that autoimmune function is closely related to malignant neoplastic diseases. Immune cells can recognise mutant proteins produced by viral and mutant genes as tumour antigens. The newly formed antigens on the surface of tumour cells are recognised by the immune system, which triggers an immune response cytotoxic T cells expressing CD8 and CD3. immune checkpoints present in immune system molecules are regulators of immune signalling and play an important role in activating T cells as well as recognising and destroying tumour cells [3]. Previously, PD-1, PD-L1, and CTLA-4 blockers have made significant breakthroughs in the immunotherapy of gastric cancer. Therefore, the search for other candidate checkpoint inhibitors is imminent. Immune cells can infiltrate gastric tissues, leading to the development of gastric malignancies. For example, HVEM is involved in tumour invasion and lymph node metastasis [4].

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Previous studies have shown that the expression of soluble HVEM in the serum of gastric cancer patients is significantly higher than that of normal controls, which is thought to be generated by shedding of the ectostructural domains rather than simple secretion [5]. In addition, the high expression of HVEM observed in gastric cancer was significantly correlated with the expression of BTLA, which is initially recognised by a variety of immune cells and thus determines the biological behaviour of cancer cells [6].

Although previous experimental and clinical studies have revealed a link between immune cells and gastric cancer. However, immune cells, especially T-cells, have become associated with increased incidence in other digestive systems [7]. However, the presence of these common risk factors may have led to bias. In addition, observational analyses may not eliminate potential confounders and unmeasured reverse causality [8]. In addition to these factors, the human and material costs invested in large samples and large-scale randomised clinical trials are enormous. Mendelian randomisation (MR) analysis, based on Mendel's law of independent distribution, is an approach that has been used in recent years mainly for epidemiological etiological inference. MR stands out as an invaluable tool in our investigation for several reasons. Firstly, by utilizing genetic variants as instrumental variables, MR offers a natural experimental setting that mimics a randomized controlled trial, thereby mitigating issues of confounding and reverse causation commonly encountered in observational studies. In the realm of immune cell research, where confounding variables can obscure true associations, MR provides a robust framework to dissect causal pathways with greater clarity. Moreover, the comprehensive two-sample MR analysis adopted in our study allows for a systematic evaluation of a wide array of immune cell signatures in relation to the risk of gastric malignancy. By leveraging genetic data associated with immune cell counts, we not only enhance the precision of our analysis but also strengthen the validity of our findings. Furthermore, the sensitivity analyses conducted within the MR framework ensure the reliability and robustness of our results, offering a rigorous approach to confirm the causal links between immune cell phenotypes and gastric cancer risk.

Genetic variation, as an instrumental variable, is subject to several assumptions. These include that the genetic variant must demonstrate a substantial and statistically significant association with the exposure variable (immune cells). In other words, the association between exposure-related genetic variants and outcome can represent the effect of exposure on outcome. Second, IV affects outcome only through exposure. Because genetic variants are randomly assigned at the time of conception, this effect is not subject to confounders or reverse causation. Thus providing another way to infer causality [9,10].

Therefore, in the present Mendelian study, we chose single nucleotide polymorphism (SNP) data from a large genome-wide association study (GWAS) of haematological traits as an instrumental variable for exposure. Previous observational studies have identified many associations between immune cells and gastric cancer. In this study, we assessed the causal relationship between immune cells and GC by a comprehensive two-sample MR approach.

Materials and Methods

Study Design

We evaluated a total of 731 causal relationships between immune cells and gastric malignancies. Based on the genetically predicted susceptibility risk of different immune cells, we assessed the causal impact of immune cells on gastric malignancy. MR uses genetic variants to represent risk factors. Therefore, valid instrumental variables (IVs) in causal inference must satisfy three key assumptions: (1) genetic variation is directly associated with exposure; (2) genetic variation is independent of possible confounders between exposure and outcome; and (3) genetic variation does not influence outcome through pathways other than exposure (Fig. 1). Studies included in our analyses were approved by the relevant institutional review boards, and participants provided informed consent.

Data Sources and Selection of Genetic Variants

We conducted an exploration in the MR Base database (http://www.mrbase.org/), a repository containing extensive summary statistics from a large number of GWASs. After quality control and imputation, this GWAS identified 1127 independent single nucleotide polymorphisms (SNPs), including more than 500 independent genomic loci at the genome-wide significance level ($p < 5 \times 10^{-8}$).

The GWAS datasets utilized in our study encompass genetic data from populations of varying ancestries, including individuals of European, Asian, and African descent. This diversity in population characteristics not only enhances the generalizability of our findings across different ethnic groups but also provides insights into potential genetic variations that may influence immune responses and gastric cancer susceptibility in distinct populations.

However, despite the strengths inherent in these GWAS datasets, certain limitations warrant consideration. Variations in sample sizes across different population groups may introduce biases or limitations in the interpretation of results, especially when exploring associations between immune cell signatures and gastric cancer risk. Additionally, the potential presence of population stratification or cryptic relatedness within the datasets could impact the validity of our MR analysis, necessitating stringent quality control measures to ensure the reliability of our findings.

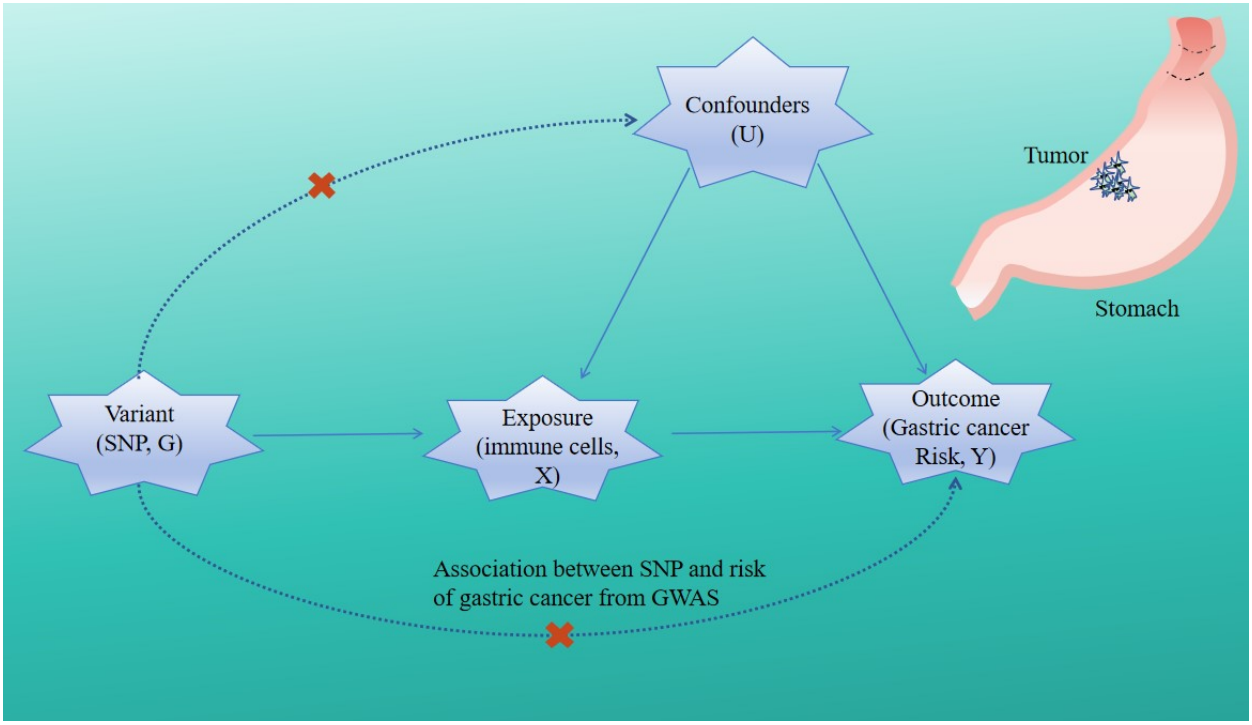

Fig. 1. Flowchart of Mendelian randomisation (MR) analysis in this study.

GWAS Data Sources for Immunisation Coverage

GWAS summary statistics for each immune trait are publicly available from the GWAS catalogue (accession numbers from GCST0001394 to GCST0002101) [11].

This Mendelian randomization study examined the causal relationships between a comprehensive set of 731 different immune phenotypes and gastric cancer risk. The immune phenotypes included in the analysis were categorized into four main groups:

1. Relative cell (RC) counts (n = 192) - Measures of the relative proportions of various immune cell types.

2. Absolute cell (AC) counts (n = 118) - Measures of the absolute numbers or concentrations of different immune cell populations.

3. Morphological parameters (MP) (n = 32) - Measures reflecting the physical characteristics and properties of immune cells.

4. Median fluorescence intensities (MFI) (n = 389) - Measures of the surface antigen levels on immune cells.

Specifically, the MFI, AC and RC features contained B cells, CDC, T cells, B cells, natural killer cells (TBNK), T cells in the maturation phase, myeloid cells, Treg cells, and monocytes, whereas the MP feature contained the TBNK panel and CDC. approximately 22 million snp were computed using a reference panel based on the Sardinian sequence [12]. Correlation tests were subsequently performed adjusting for covariates (i.e., age, sex and age2). It is worth noting that the initial GWAS of immune profiles utilised data from European individuals with no overlapping cohorts.

Selection of Instrumental Variables (IVs)

The significance level for each immune trait was set at 1×10^{-5} according to a recent study [13]. We clipped these SNPs (linkage disequilibrium clustering (LD) r2 threshold = 0.001 at a distance of 10 kb) using the clumping procedure in PLINK software (v1.90) [14]. Meanwhile, in the palindromic region, the minor allele frequency of snp was allowed to be 0.3. Subsequently, we extracted statistics related to the associations between these genetic variants and GC using the results of a more relaxed clustering threshold (R2 <0.01). For GC, we adjusted the significance level to 5×10^{-8}. The proportion of phenotypic variance explained (PVE) and the F statistic were calculated for each IV to assess IV strength and avoid weak instrumental bias. Then, after excluding IVs with low (<10) F-statistics, 1127 IVs with GC were retained for further analysis. Finally, the MR-Egger intercept was used to identify and exclude multivalent outliers.

The rationale behind the specific threshold values used in SNP selection lies in the need to identify genetic variants strongly associated with immune traits while minimizing false positives. By setting stringent significance thresholds, such as a *p*-value of 1×10^{-5}, we aim to prioritize SNPs that exhibit a high level of statistical significance in their association with immune cell phenotypes. This approach helps reduce the likelihood of including spurious or weakly associated SNPs in our analysis, thus enhancing the precision and validity of our MR study.

Furthermore, the method of LD clumping is employed to address the issue of linkage disequilibrium, where ge-

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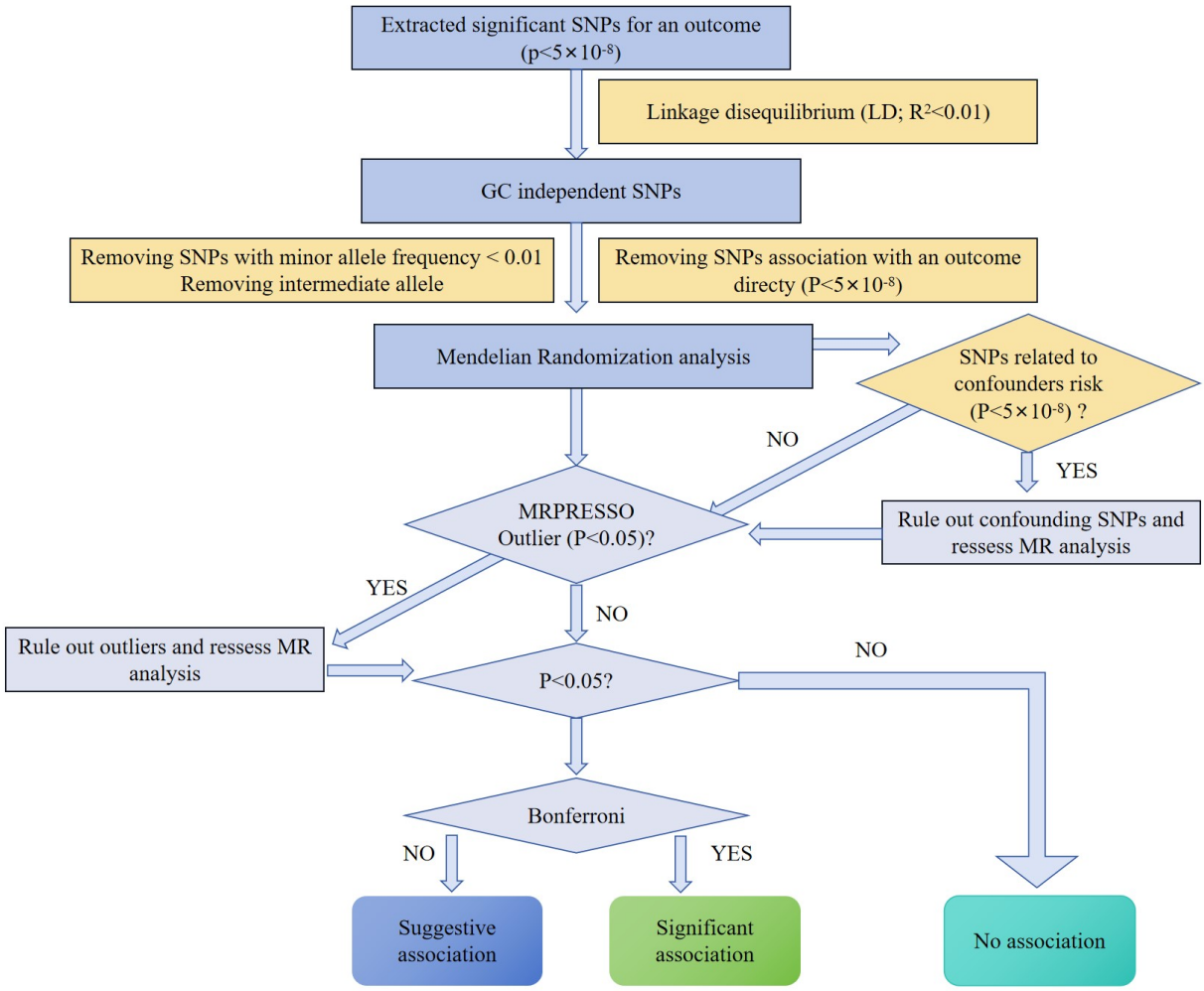

Fig. 2. Flow chart about the analytical methods and how the MR analysis was performed.

netic variants in close proximity on the DNA strand tend to be inherited together. LD clumping allows us to select a subset of independent SNPs that capture the genetic variation within a specific genomic region, thereby avoiding redundancy and ensuring that the selected SNPs represent distinct genetic signals rather than redundant information.

Data Analysis

All analyses were performed in R version 4.0.2 software (http://www.Rproject.org). In order to assess the causal relationship between the 731 immunophenotypes and GC, the inverse variance weighted, weighted median and simple mode were mainly performed using the 'MendelianRandomization' package (version 0.4.3) software package [15–17]. Heterogeneity between the selected iv was tested using Cochran's Q statistic and the corresponding *p*-value. If the original hypothesis was rejected, a random effects IVW was used instead of a fixed effects IVW [18]. The proportion of variance explained by each IV (PVE) was used to explain the strength of the selected SNPs and was calculated as PVE = 2 × EAF × (1 – EAF) × β^2. Instrument strength was then assessed using the F statistic. This statistic reflects the exposure variance explained by the instrumental variables. Calculation of the F statistic was based on the PVE value, i.e., [PVE × (n – 1 – k)]/[(1 – PVE) × k]. (EAF, effect allele frequency; β, effect size on exposure; n ,effective sample size of exposed GWAS, and k represents the number of variants included in the IV model). To determine the power of the MR results, we used an online calculator (https://shiny.cnsgenomics.com/mRnd/) to make power estimates from a given type I error rate (α 0.05) and the OR of the IVW estimate (Fig. 2). To exclude the effect of horizontal multiplicity, we used a commonly used method (i.e., MR-Egger), which states that horizontal multiplicity exists if its intercept term is statistically significant [19]. In addition, we used funnel plots and scatter plots. Funnel plots show that the correlations are robust and free of heterogeneity. Scatter plots show that the results are not affected by outliers.

The power of an MR study was calculated using the following formula: Power = $1 - \beta$

where \beta$\beta$ represents the probability of a Type II error, which is the likelihood of failing to reject a false null hypothesis (i.e., not detecting a true causal effect). Power calculations often involve estimating the effect size of the exposure on the outcome, the standard error of the effect estimate, and the desired level of statistical significance. To perform power calculations for our MR analysis investigating the causal relationship between immune cells and gastric cancer risk, we considered the effect sizes of the genetic variants on immune traits, the expected effect sizes on gastric cancer risk, and the sample size of our study population. By conducting power calculations, we can determine the statistical power of our analysis and ensure that our study is adequately powered to detect significant causal relationships.

The F statistic is a key metric used in MR studies to assess the strength of the genetic instruments employed in the analysis. In the context of MR, a high F statistic indicates a strong instrument that is associated with the exposure variable (e.g., immune cell phenotype) and is independent of potential confounders. The F statistic is calculated using the following formula: $F = (N - k - 1)R^2/k(1 - R^2)$, where: N is the sample size. k is the number of instrumental variables. R^2 is the proportion of variance in the exposure variable explained by the instrumental variables.

A high F statistic (greater than 10) indicates that the instrumental variables have a strong explanatory power for the exposure variable, which enhances the validity of the MR analysis and strengthens causal inference. In our study, we calculated the F statistic for our genetic instruments related to immune cell phenotypes to evaluate the strength of the instruments and assess the reliability of our MR analysis in determining causal relationships between immune cells and gastric cancer risk.

Results

Immune Cell Effects on Gastric Cancer (GC)

Protective Immune Phenotypes

In the analysis investigating the impact of immune cell phenotypes on gastric cancer, several immune cell types demonstrated a protective effect against gastric malignancies. Notably, the following immune cell types were found to be associated with a reduced risk of gastric cancer:

Naive-mature B cell %B cell: OR = 0.82, 95% CI [0.70–0.95], $p = 0.012$

IgD+ CD24- %lymphocyte: OR = 0.71, 95% CI [0.58–0.86], $p = 0.003$

Resting Treg AC: OR = 0.65, 95% CI [0.50–0.84], $p = 0.002$

CD33- HLA DR+ AC: OR = 0.92, 95% CI [0.78–1.08], $p = 0.31$

CD66b++ myeloid cell AC: OR = 1.14, 95% CI [1.01–1.29], $p = 0.028$

Naive CD4+ AC: OR = 0.77, 95% CI [0.63–0.93], $p = 0.008$

Risk-associated Immune Phenotypes

The estimate for the ratio of IgD+ CD24- %lymphocyte to gastric cancer risk (OR) was found to be 0.8938 (95% CI = 0.8323–0.9598) with a p-value of 0.0020 using the IVW method. Similar results were replicated when employing the Weighted Median and Weighted Mode methods. With the Weighted Median method, the OR was 0.8840 (95% CI = 0.7977–0.9796) with a p-value of 0.0186. Likewise, the Weighted Mode method yielded an OR of 0.8702 (95% CI = 0.7699–0.9837) with a p-value of 0.0368. In contrast, the Simple Mode analysis did not identify a correlation between immune cells and gastric malignancy, with an OR of 0.8816 (95% CI = 0.7596–1.0232) and a p-value of 0.1115. Employing the same methodology, we made predictions regarding the association between immune cells and gastric cancer risk. Fig. 3 provides a comprehensive summary of the heterogeneity, multiplicity, and sensitivity analyses associated with immune cells and gastric cancer risk.

Gastric Cancer Effects on Immune Cells

Increased Immune Cell Types in Gastric Cancer Patients

In the examination of how gastric cancer influences immune cell populations, several immune cell types were found to be elevated in gastric cancer patients compared to controls. The following immune cell types exhibited increased levels in individuals with gastric cancer: IgD+ CD38dim AC, IgD- CD27- AC, Memory B cell %B cell, IgD- CD24- AC, IgD+ CD24- AC, CD20- AC, CD11c+ CD62L- monocyte AC, CD62L- HLA DR++ monocyte AC, CD11c+ HLA DR++ monocyte AC, Myeloid DC %DC, HLA DR++ monocyte %leukocyte, HSC AC, DN (CD4-CD8-) AC, DN (CD4-CD8-) %leukocyte, CD8br NKT %lymphocyte, CD24 on IgD- CD38-, HVEM on TD CD8br, CD28 on CD39+ resting Treg, PDL-1 on CD14-CD16+ monocyte, SSC-A on HLA DR+ NK, SSC-A on CD4+, SSC-A on HLA DR+ CD8br (Table 1).

Decreased Immune Cell Types in Gastric Cancer Patients

Conversely, the remaining immune cell types showed decreased expression levels in patients with gastric cancer. These findings suggest potential alterations in the immune landscape associated with gastric malignancies.

The two-sample Mendelian randomization analysis indicated significant alterations in immune cell populations in individuals with gastric cancer, with specific immune cell types showing both elevations and reductions, indicating a complex immune response to gastric cancer progression.

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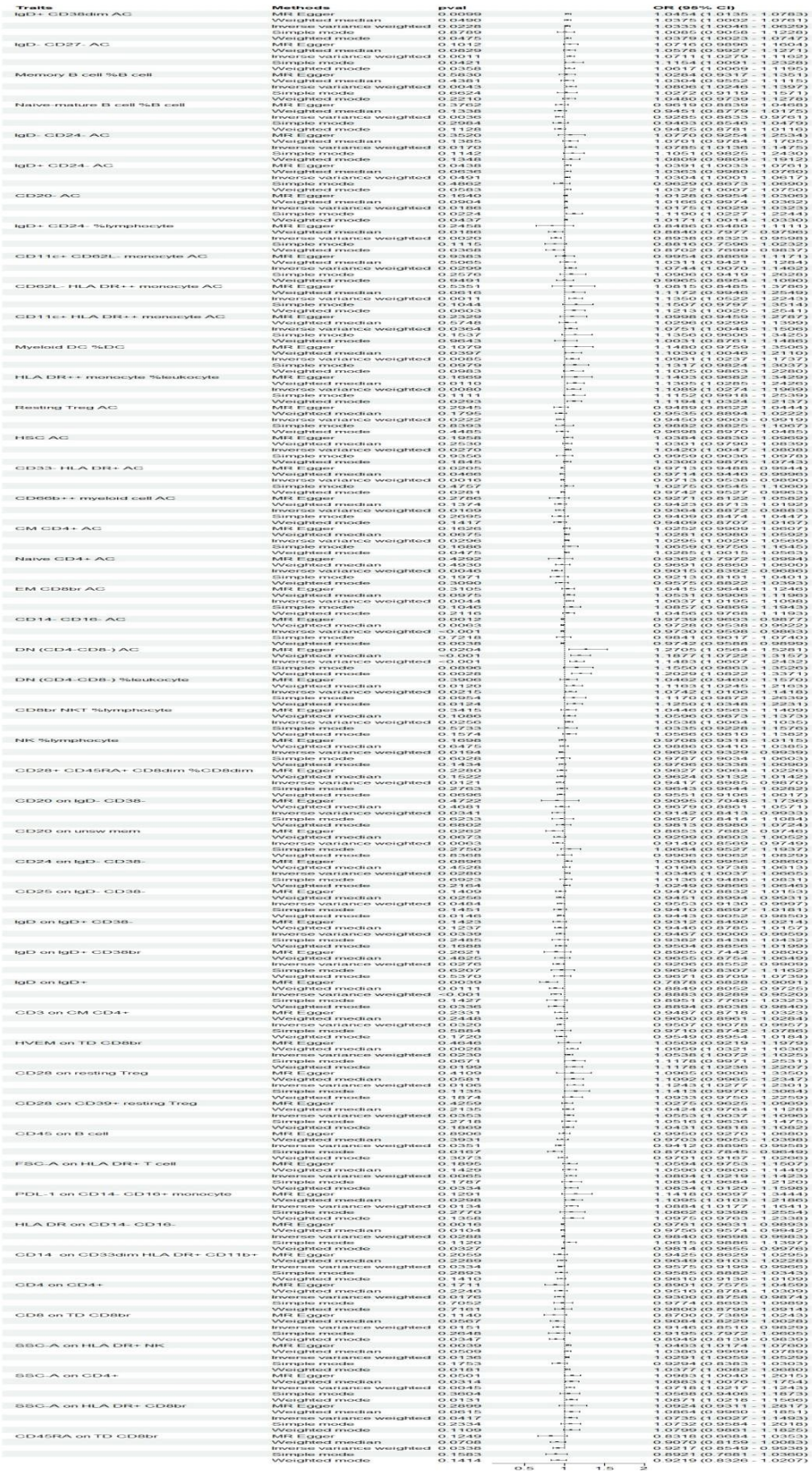

Fig. 3. Mendelian randomization estimates of the association between immune cells and risk of gastric cancer. OR, odds ratio; CI, confidence interval.

Table 1. Mendelian randomization results for the relationship between gastric cancer and immune cells.

ID	Immune cell	Methods	*p*
ebi-a-GCST90001394	IgD+ CD38dim AC	All SNPs-IVW	0.022785079
ebi-a-GCST90001401	IgD- CD27- AC	All SNPs-IVW	0.001091321
ebi-a-GCST90001406	Memory B cell %B cell	All SNPs-IVW	0.004323648
ebi-a-GCST90001408	Naive-mature B cell %B cell	All SNPs-IVW	0.003611445
ebi-a-GCST90001414	IgD- CD24- AC	All SNPs-IVW	0.016983378
ebi-a-GCST90001416	IgD+ CD24- AC	All SNPs-IVW	0.049068633
ebi-a-GCST90001421	CD20- AC	All SNPs-IVW	0.018638787
ebi-a-GCST90001441	IgD+ CD24- %lymphocyte	All SNPs-IVW	0.002015424
ebi-a-GCST90001452	CD11c+ CD62L- monocyte AC	All SNPs-IVW	0.029887331
ebi-a-GCST90001454	CD62L- HLA DR++ monocyte AC	All SNPs-IVW	0.001054597
ebi-a-GCST90001456	CD11c+ HLA DR++ monocyte AC	All SNPs-IVW	0.036411177
ebi-a-GCST90001459	Myeloid DC %DC	All SNPs-IVW	0.008459905
ebi-a-GCST90001476	HLA DR++ monocyte %leukocyte	All SNPs-IVW	0.007967962
ebi-a-GCST90001480	Resting Treg AC	All SNPs-IVW	0.022176734
ebi-a-GCST90001514	HSC AC	All SNPs-IVW	0.027023856
ebi-a-GCST90001523	CD33- HLA DR+ AC	All SNPs-IVW	0.001637577
ebi-a-GCST90001529	CD66b++ myeloid cell AC	All SNPs-IVW	0.016883126
ebi-a-GCST90001537	CM CD4+ AC	All SNPs-IVW	0.029619584
ebi-a-GCST90001540	Naive CD4+ AC	All SNPs-IVW	0.004605927
ebi-a-GCST90001554	EM CD8br AC	All SNPs-IVW	0.004354375
ebi-a-GCST90001581	CD14- CD16- AC	All SNPs-IVW	8.12×10^{-05}
ebi-a-GCST90001598	DN (CD4-CD8-) AC	All SNPs-IVW	0.000640958
ebi-a-GCST90001613	DN (CD4-CD8-) %leukocyte	All SNPs-IVW	0.021492144
ebi-a-GCST90001632	CD8br NKT %lymphocyte	All SNPs-IVW	0.025611979
ebi-a-GCST90001647	NK %lymphocyte	All SNPs-IVW	0.019427434
ebi-a-GCST90001665	CD28+ CD45RA+ CD8dim %CD8dim	All SNPs-IVW	0.012149292
ebi-a-GCST90001755	CD20 on IgD- CD38-	All SNPs-IVW	0.03409717
ebi-a-GCST90001760	CD20 on unsw mem	All SNPs-IVW	0.006259089
ebi-a-GCST90001769	CD24 on IgD- CD38-	All SNPs-IVW	0.028045066
ebi-a-GCST90001787	CD25 on IgD- CD38-	All SNPs-IVW	0.048352903
ebi-a-GCST90001822	IgD on IgD+ CD38-	All SNPs-IVW	0.033921961
ebi-a-GCST90001824	IgD on IgD+ CD38br	All SNPs-IVW	0.027625325
ebi-a-GCST90001827	IgD on IgD+	All SNPs-IVW	0.000796866
ebi-a-GCST90001841	CD3 on CM CD4+	All SNPs-IVW	0.031993296
ebi-a-GCST90001874	HVEM on TD CD8br	All SNPs-IVW	0.023034063
ebi-a-GCST90001900	CD28 on resting Treg	All SNPs-IVW	0.01061661
ebi-a-GCST90001901	CD28 on CD39+ resting Treg	All SNPs-IVW	0.035261955
ebi-a-GCST90001910	CD45 on B cell	All SNPs-IVW	0.035082655
ebi-a-GCST90001975	FSC-A on HLA DR+ T cell	All SNPs-IVW	0.006523149
ebi-a-GCST90001999	PDL-1 on CD14- CD16+ monocyte	All SNPs-IVW	0.013406277
ebi-a-GCST90002009	HLA DR on CD14- CD16-	All SNPs-IVW	0.028776701
ebi-a-GCST90002021	CD14 on CD33dim HLA DR+ CD11b+	All SNPs-IVW	0.033364763
ebi-a-GCST90002022	CD4 on CD4+	All SNPs-IVW	0.017649879
ebi-a-GCST90002057	CD8 on TD CD8br	All SNPs-IVW	0.015069681
ebi-a-GCST90002077	SSC-A on HLA DR+ NK	All SNPs-IVW	0.01363548
ebi-a-GCST90002081	SSC-A on CD4+	All SNPs-IVW	0.004522688
ebi-a-GCST90002086	SSC-A on HLA DR+ CD8br	All SNPs-IVW	0.041675851
ebi-a-GCST90002101	CD45RA on TD CD8br	All SNPs-IVW	0.033780828

AC, absolute count.

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Discussion

Combined with PubMed search results, this article is the first MR analysis of causal relationships between multiple immune phenotypes and GC. Based on the large amount of publicly available genetic data, we explored the causal relationship between 731 immune cell traits and gastric cancer. The results of this Mendelian randomisation study suggest that dozens of immune cell phenotypes have a causal effect with gastric malignancies. However, when the exposure factor was changed to gastric malignancy and the outcome event to immune cell phenotype, we found a gastric causal association between the two (p value > 0.05).

In recent years, a large number of studies have highlighted the extensive role of immune cells in cellular carcinogenesis and described them as key regulators in the field of inflammation and digestion [20–22]. In the development of gastric cancer, it is also closely related to differences in the proportion of different immune cells. Naive CD4+% T cells are able to regulate pro- and anti-inflammatory signals by differentiating into multiple helper T cell (Th) cell lines, each with its own unique cytokine profile and function. In recent years, some experiments have shown that secreted cytokines, such as IL-6, CD8+ T cells, and immune cells such as CD4+ T cells, play an important role in gastric cancer development, progression, and even metastasis [23–25].

Activated and quiescent CD24- %lymphocyte has also been shown to be significantly associated with the risk of gastric malignancy [26]. Furthermore, it is noteworthy that the presence of gastric malignancy was associated with IgD+ CD38dim AC, IgD- CD27 AC, Memory B cell %B cell, CD11c+ CD62L- monocyte AC, DN (CD4-CD8-)AC, DN (CD4-CD8-)%leukocyte, HVEM on TD CD8br, FSC-A on HLA DR+ T cell, and other immune cell levels correlated with the increase. In addition, several studies have shown that patients with gastric malignancies have increased concentrations of inflammatory cytokines in the blood [27,28]. The ligand HVEM (also known as TNFRSF14) is a member of the tumour necrosis factor receptor (TNFR) superfamily. HVEM is widely present in haematopoietic cells and in various parenchymal cells, such as breast, melanoma, gastric, and ovarian cancer cells [29–32]. HVEM has emerged as a bi-directional immune molecule, which, by binding to BTLA or LIGHT involved in suppressing or stimulating T cells (TNFSF14). In recent years, the BTLA/HVEM pathway is a new immune escape route that has been suggested to be a key factor in the physiological processes of inflammation and tumourigenesis [33].

Mechanisms underlying causal relationships:

1. **Immune surveillance and tumor recognition**:
   - **Tumor antigen recognition**: Immune cells, especially T-cells, play a pivotal role in recognizing and targeting mutant proteins produced by tumor cells as antigens. This recognition triggers an immune response mediated by cytotoxic T cells expressing CD8 and CD3, leading to tumor cell destruction.
   - **Immune checkpoints**: Molecules like PD-1, PD-L1, and CTLA-4 act as immune checkpoints regulating immune signaling. In GC, inhibitors targeting these checkpoints have shown promise in immunotherapy. The study potentially highlights the importance of these checkpoints in GC pathogenesis.
2. **Inflammation and tumor microenvironment**:
   - **Infiltration of immune cells**: Immune cells infiltrating gastric tissues can promote tumorigenesis. For instance, the involvement of molecules like HVEM in tumor invasion and metastasis underscores the influence of immune cell interactions in GC progression.
   - **Immune cell subtypes**: The study identifies specific immune cell subtypes associated with GC risk, suggesting a nuanced interplay between different immune cell populations and the tumor microenvironment.

Implications for understanding GC:

1. **Therapeutic opportunities**:
   - **Immunotherapeutic targets**: Identification of immune cell types causally linked to GC risk provides potential targets for immunotherapy. Understanding the specific immune responses associated with GC can guide the development of novel treatment strategies.
   - **Checkpoint inhibitors**: Insights into the immune landscape of GC can inform the selection of checkpoint inhibitors and personalized immunotherapies tailored to the immune profiles of individual patients.
2. **Risk prediction and stratification**:
   - **Biomarkers for risk assessment**: Immune cell signatures associated with GC risk could serve as biomarkers for predicting susceptibility to the disease. Integrating immune cell profiles into risk assessment models may enhance early detection and personalized management of GC.
3. **Research directions**:
   - **Further investigations**: The study underscores the need for continued research into the complex interplay between immune cells and GC. Future studies could delve deeper into understanding the molecular mechanisms driving immune responses in GC and explore novel therapeutic avenues.

This study is based on two-sample MR analyses using a large published GWAS cohort and results in high statistical efficiency. The conclusions of this study are based

on genetic instrumental variables, and causal inference was performed using multiple MR analyses. The results are robust and not confounded by horizontal pleiotropy and other factors. However, our study has some limitations that require further improvement. The study is confronted with numerous sources of bias and limitations that necessitate meticulous consideration. By confining the research exclusively to European populations for immune trait GWAS data, there is a risk of introducing population-specific biases, potentially impeding the generalizability of findings to other ethnic groups characterized by distinct genetic backgrounds and varying environmental exposures. The MR approach, essential in this study, assumes that genetic variants do not correlate with confounding factors that could influence both immune cell levels and the risk of GC. Unaccounted confounders, if present, could introduce bias and compromise the accuracy of causal inferences drawn from the study. The validity of instrumental variables, a cornerstone of MR analysis, is critical. Any violations of the assumptions underlying these instrumental variables could result in biased estimates of causal effects. Moreover, the study's exclusive focus on European populations raises concerns regarding the transferability of results to populations with diverse genetic backgrounds and immune cell profiles. Challenges related to the quality and heterogeneity of GWAS data for immune traits could introduce noise and bias, necessitating thorough sensitivity analyses to enhance the reliability of results. The potential for heterogeneity and pleiotropy in genetic instruments used in MR analysis poses additional risks of biased estimates, underlining the importance of addressing these issues through robust statistical methods. Furthermore, the presence of bidirectional relationships between immune cells and GC, where GC may also influence immune cell profiles, underscores the necessity of accounting for potential reverse causation effects. Ethical considerations, such as obtaining proper ethical approval and ensuring informed consent for the studies included in the analysis, are integral to upholding the integrity of the research. To advance future research in this field, it is recommended to incorporate diverse populations in studies to improve the external validity of findings and consider the genetic and environmental heterogeneity across different ethnic groups. Conducting comprehensive sensitivity analyses, leveraging multi-ethnic GWAS data for immune traits, and fostering collaborative research efforts are essential steps to enhance the reliability and generalizability of study findings regarding the intricate relationship between immune cells and GC.

Conclusion

The study conclusively identifies specific immune cell phenotypes associated with gastric cancer risk. Naive-mature B cell %B cell, IgD+ CD24- %lymphocyte, and Resting Treg AC are highlighted as protective factors against gastric malignancies. Conversely, CD66b++ myeloid cell AC is linked to an elevated risk of gastric cancer. Furthermore, the ratio of IgD+ CD24- %lymphocyte is significantly correlated with gastric cancer risk across various statistical methods. These findings underscore the dynamic interplay between immune responses and gastric cancer progression, highlighting the potential for tailored immunotherapeutic strategies based on individual immune profiles. The observed alterations in immune cell populations in gastric cancer patients unveil a complex immune landscape, emphasizing the need for further research to elucidate the underlying mechanisms and explore novel therapeutic avenues in the field of gastric cancer treatment.

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